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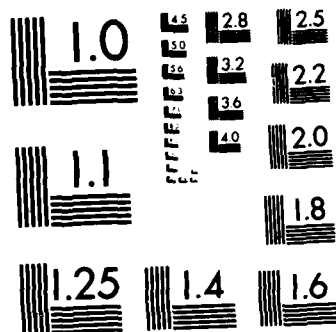
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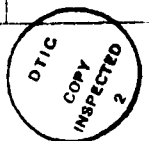
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International Association for Dental Research, 60th General Session,
New Orleans, Louisiana, March 18-21, 1982.

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*Author presenting paper.

Longevity of Amalgam Restorations: A Retrospective View. J. C. CECIL*, M. E. COHEN, D. C. SCHROEDER, S. L. TAYLOR. Naval Dental Research Institute, Great Lakes, IL 60088

The purpose of this study was to describe the natural history of amalgam restorations placed in teeth of young adults (ages 17-29 years) as a function of tooth type and surface coverage. The unit of measure was the first 1-, 2-, 3-, and 4+-surface amalgam restoration identified in 300 randomly selected dental records. Restorations were followed through the dental record to determine changes in surface coverage or other outcomes -- extraction, endodontic therapy, crown placement -- which occurred over time. The lifespan of a restoration was considered to be the interval between initial placement and date of subsequent change or date of most recent examination. Measurements of lifespan were therefore values of minimal longevity. The mean lifespan for 831 total restorations was 4.25 yrs; 1-surface=4.53, 2-surface=4.51, 3-surface=4.07, and 4+-surface=3.14 years. The range for the various types of restorations was zero to 20 years of service without change. There were no differences in longevity for restorations placed in anatomically different teeth (i.e., molars vs premolars). Restorations which changed (N=152, 18%) had a mean lifespan of 3.22 years; 122 (or 80%) of these were replaced, repaired, or had additional surfaces placed within a four-year period. Of the 831 restorations, 303 (or 36%) exceeded the mean minimum-lifespan of 4.25 years. Only 12 (4%) of the restorations in the study resulted in extraction of the tooth. It was concluded that one might expect amalgam restorations placed under the clinical conditions of this study to have a minimal-lifespan of four years.

Supported by NMRDC Project No. MF58.524.012-0030.

Conservative Treatment of Deep Carious Lesions in Naval Recruits.
G. E. CLARK*, R. G. WALTER and R. G. ESQUIRE. Naval Dental Research
Institute, Great Lakes, IL 60088

A study in naval recruits is in progress to establish reliable methods of conservative treatment for deep carious lesions. The methods evaluated were: all caries removed with no pulp exposure (ACR), indirect pulp cap (IPC), and direct pulp cap (DPC). All treatment methods included permanent restorations. Teeth with caries which have penetrated at least 3/4 through the dentin thickness as determined by radiographs were included in the study. Clinical criteria such as history of symptoms, periapical radiographs, and diagnostic electric, cold, percussion and heat tests were recorded for all teeth prior to treatment and at yearly recall intervals. Materials and methods were recorded subsequent to restoration of the teeth. Clinical criteria associated with treatment failure are: presence of osteosclerosis and/or enlargement of periodontal ligament space; pain duration greater than one hour and spontaneous or severe pain; no response to diagnostic test; and retreatment by extraction for pulpal reasons, replacement of restoration and root canal therapy. Data were available for one or more annual recall intervals for 200 teeth. Treatment for recall years one through four was about 85% successful for the methods of ACR, IPC, and DPC. Chi square tests of the data indicated there were no significant differences among the ACR, IPC, and DPC treatments and recall years. Success was not dependent on the type of conservative treatment. Restoration of deep carious lesions by ACR, IPC, and DPC appear to be reliable methods of treatment for at least four years.

Supported by NMRDC Project No. M0095PN003.3008.

Dental History Predictors of Caries Related Dental Emergencies.
M. E. COHEN* and J. C. CECIL. Naval Dental Research Institute,
Great Lakes, Illinois 60088

The identification of potential dental emergencies among military personnel could decrease the likelihood that missions will be compromised or aborted. Research was therefore directed towards the development of predictive models for the presence of deep carious lesions (treatment need) and for emergency dental visits because of caries (demand for treatment). The population investigated consisted of naval personnel who visited a Naval Regional Dental Center because of (1) caries related pain, (2) routine examination, or (3) response to a recall notice (N=50/gp). Information pertaining to 16 potential predictors of a behavioral (viz temporal patterns of preventive and treatment appointments) or a clinical (extent of past disease and past treatment) nature was obtained from the dental records. Step-wise regression revealed that two variables could account for 30% of the variation in emergency vs non-emergency visit classification. A single variable, number of carious surfaces not treated within three months of the last examination, could account for 67% of the variation in number of deep lesions. Contrary to usual expectations, the time interval since the last dental appointment was unrelated to the dependent variables. Many individuals who did not seek routine examinations failed to develop disease while others obtained only palliative treatment for frequent episodes of acute pain. The surfaces-not-treated variable, if used prospectively to select individuals at high risk for deep lesions would exhibit at one criterion level, sensitivity of 0.90 and specificity of 0.82.

Supported by NMRDC 61152N MR00001.0027.

Plaque Fluoride and Microbial Levels in Response to .05% NaF Rinsing.
R. G. ESQUIRE*, I. L. SHKLAIR, J. C. CECIL and G. E. CLARK. Naval
Dental Research Institute, Great Lakes, IL 60088

The purpose of this study was to determine the effects of daily rinsing with 0.05% NaF (225 ppm F^-) on plaque fluoride (F) and microbial content in young adults. These criteria were hypothesized to provide indirect estimates of an anti-carries effect. Naval recruits (186 males of mean age 20 ± 2.8 S.D.) were randomly selected for a double-blind study involving daily rinsing with either a 0.05% NaF or a placebo solution for 18 weeks. Posterior interproximal plaque was assayed for bound and unbound F, S. mutans, Lactobacilli and total aerobes, at 6-week rinsing intervals. Mean baseline F values were: total F = 6.7 ± 7.1 ng/mg plaque (wet weight), which consisted of 4.5 ± 4.3 ng/mg unbound F and 2.2 ± 4.2 ng/mg bound F. Mean baseline microbial counts were: S. mutans = $8.8 \pm 22.3 \times 10^4$ CFU/mg, Lactobacillus = $3.6 \pm 17.1 \times 10^3$ CFU/mg, and total aerobes = $8.5 \pm 13.6 \times 10^6$ CFU/mg. A significantly higher ($p < .05$) total fluoride level was noted in the fluoride-rinse (FR) group, 11.1 ± 10.7 ng/mg, compared with the placebo-rinse (PR) group, 8.3 ± 7.9 ng/mg, but only at the 12th week of rinsing. This contrast was attributable to a significant difference ($p < .05$) in the unbound F component, 7.8 ± 8.2 ng/mg (FR) vs 5.7 ± 4.9 ng/mg (PR). All other intergroup comparisons for F were not significantly different. No significant differences were shown between groups in any of the microbial counts. There was a high degree of individual variation in plaque fluoride and microbial content. Daily rinsing with 0.05% NaF after 18 weeks had no effect on plaque F and microbial content in young adults.

Supported by NMRDC 62758N MF58.524.012.0028.

Salivary Peroxidase System Components in Caries-Free and Caries-Active Subjects. B. L. LAMBERTS*, E. D. PEDERSON, M. P. GOLDING, and K. M. PRUITT, NDRI, Great Lakes, IL 60088 and U. of AL in Birmingham, 35294

The aims of this study were (a) to determine whether levels of the salivary antibacterial agent, hypothiocyanite (OSCN^-), differed in caries-free (CF), DMFT=0, and caries-active (CA), D>5, Navy recruits, and (b) to investigate interrelations among factors affecting OSCN^- production in these subjects. Unstimulated whole saliva was collected in chilled containers from 29 CF and 29 CA recruits, along with data on smoking habits. Flow rate, pH, OSCN^- , thiocyanate (SCN^-), and lactoperoxidase activity (LPO) were determined for each sample. Comparative data (mean \pm s.d.) were obtained as follows: Flow rates (ml/min)= 0.29 ± 0.21 , CF, vs 0.29 ± 0.19 , CA; pH= 6.88 ± 0.27 , CF, vs 6.91 ± 0.29 , CA; OSCN^- (μM)= 47.1 ± 26.0 , CF, vs 49.3 ± 24.0 , CA; SCN^- (mM)= 1.46 ± 0.86 , CF, vs 1.45 ± 0.73 , CA; LPO (mU/ml)= 123.9 ± 66.0 , CF, vs 168.3 ± 101.9 , CA. None of these comparisons showed statistically significant differences. Correlations among these variables were then examined for the 58 subjects, which included 34 smokers. Although OSCN^- showed no correlation with SCN^- , both OSCN^- and SCN^- showed negative correlations ($p < .05$) with LPO. Flow rates and pH were also negatively correlated with SCN^- ($p < .05$). Positive correlations were found between smoking and levels of OSCN^- and of SCN^- ($p < .01$), along with a negative correlation ($p < .01$) of smoking and LPO.

Supported by NMRDC F58524.MR0412002-0443.

History of Retention and Extraction of Third Molars in Naval Personnel. D. C. SCHROEDER*, J. C. CECIL and M. E. COHEN. Naval Dental Research Institute, Great Lakes, IL 60088

The risks involved in retaining third molars are controversial. The purpose of this study was to describe the history of extracted and retained third molars in naval personnel. Dental records of 300 randomly selected staff personnel stationed at the Great Lakes Naval Base were examined. Mean age of the sample at time of entry into the Navy was 19.6 years. There were 1072 third molars present and 646 of these were unerupted. Both crown encapsulation and clinical angulation were determined for unerupted third molars. For maxillary molars, 91.1% were vertically or distoangularly inclined and 79.6% of mandibular third molars were vertically or mesioangularly inclined. During the period under study 185 third molars erupted. The most common reason given for extracting mandibular third molars was "Malposition" and for maxillary third molars was "No Opposing Tooth." Mean age of extraction was 23.4 years. Third molar extraction was directly related to whether a tooth had erupted. Each quadrant was evaluated separately and third molars were placed into one of three categories; unerupted, erupted after entry, or erupted at entry. Using a 2x3 chi square, the differences were statistically significant for all four quadrants at the $p < 0.02$ level. Third molars were less likely to have been extracted if they were erupted initially or erupted during the study. Extraction was not related to crown encapsulation or angulation. The overall rate of extraction decreased with age while the reason for extraction was more likely to be related to symptomatic conditions as age increased.

Supported by NMRDC Project No. MR000.01.0029.

The Effect of Marine and Fresh-Water Atmospheric Environments on
Packaged Dental Instruments. A. SEROWSKI* and F. AKER. Naval
Dental Research Institute, Great Lakes, IL 60088

Previous study has shown that marine atmospheric environments produce significant amounts of corrosion and deterioration on dental instruments in a 30-day period. The purpose of this study was to determine if dental instruments packaged in paper and plastic sterilization bags would corrode or deteriorate in marine and fresh-water atmospheric environments; conditions frequently encountered during marine and naval operations. Selected dental instruments were placed in paper and plastic sterilization bags, sealed, and then placed in two high-humidity chambers for 30 days; one chamber represented the marine (salt water) environment and the other a fresh water environment. Each item was categorized by manufacturer, metal(s), packaging and test environment, and was examined macroscopically and microscopically at the end of the test period. Both the percent of surface area affected by corrosion and a description of the deterioration were recorded. Dental instruments sealed in plastic bags showed no corrosion. Dental instruments placed in paper bags showed less than two percent corrosion. All dental instruments in either plastic or paper sterilization envelopes were considered usable after exposure. These observations indicate that protecting dental instruments in paper or plastic sterilization bags is an effective method of deterring the detrimental effects of corrosion from both marine and fresh-water atmospheric environments over a 30-day period.

Supported by NMRDC 63706N M0095-PN.003-3017.

The Effect of Three Surfactants on Controlling Caries Activity in Hamsters. I. L. SHKLAIR*, R. W. GAUGLER, R. G. WALTER. Naval Dental Research Institute, Great Lakes, IL 60088

We previously reported (IADR Abstract #559, 1981) that three surfactants, benzathonium chloride (BC), cetylpyridinium chloride (CPC) and sodium lauryl sulfate (SLS), at low concentrations, inhibited in vitro the glucosyltransferase activity of Streptococcus mutans. In the present study these 3 compounds were tested to determine if they could prevent or reduce dental caries in a hamster caries model system. The hamsters were infected with S. mutans 6715 and fed diet 2000. The animals were swabbed 3 times a week with a 0.05% solution of either BC, CPC, or SLS. The organism was tested for its ability to synthesize soluble and insoluble glucans at the time of initial implantation and at the end of the experiment. At day 60 the animals were killed and their caries scores determined by the method of Keyes. The positive control and the test animals were carriers of S. mutans at day 60. The BC treated group had significantly lower caries scores ($p < 0.05$) than the positive control or the other test groups. Although the BC treated animals retained high oral levels of S. mutans, the soluble and insoluble glucans synthesized by S. mutans isolated from the BC treated group at the end of the experiment was significantly lower ($p < 0.001$) than when the organisms were initially implanted. The glucans synthesized by S. mutans in the CPC and SLS groups were not significantly different ($p > 0.05$) at the beginning than at the end of the experiment. The reduction in glucans synthesized by the S. mutans in the BC treated group appeared to account for the lowered caries scores in this group.

Supported by NMRDC 61153N MR0412002.0441.

Effect of Saliva and Sucrose on Adherence of *S. mutans* to Insoluble Streptococcal Glucan. L. SIMONSON*, B. LAMBERTS, E. PEDERSON and D. REIHER. Naval Dental Research Institute, Great Lakes, IL 60088

A new model was developed to study cell-to-cell accumulation. The assay measures the quantitative adherence of ^3H -thymidine labelled oral streptococci to uniformly sized disks of water-insoluble glucan. The disks were prepared from KOH soluble, but water-insoluble glucan synthesized by cell-free culture supernatants of *Streptococcus mutans* strain K1R. The disks were pretreated with pooled whole saliva from either caries-free (CF) (DMFT=0) or caries-active (CA) (DT>5) naval recruits, and incubated in a neutral buffer with and without 4% sucrose. Washed glucose-grown cells from serotypes b, c, d/g and E attached to the glucan disks at significantly greater ($p<.001$) levels than disks of the same size made of hydroxyapatite. There was a significant increase ($p<.001$) in attachment to the glucan disks in the presence of sucrose for serotype a and to a greater extent by serotype d/g cells (the same serotype used to prepare the glucan disks). The serotype d/g cell adherence to the glucan disks was also significantly greater ($p<.001$) than that of other serotypes in the absence of sucrose. Precoating the glucan disks with either CA or CF saliva had no effect on adherence of any of the *S. mutans* serotypes studied relative to untreated controls. The greater adherence of serotype d/g cells to serotype d/g glucan disks with and without sucrose could either reflect specific recognition or a nonspecific function related to greater production of insoluble glucan by this serotype. Saliva was not a significant factor affecting this cell-to-cell accumulation.

Supported by NMRDC Project No. MF58524012.0027.

The purpose of this investigation was to determine the effect of a low molecular weight dextran, T-10, on caries development and plaque accumulation in 20-day-old Osborne-Mendel derived rats and 17- and 21-day-old Golden hamsters using various feeding regimens. The animals were inoculated with Streptococcus mutans 6715 and maintained on one of two cariogenic diets: (1) diet 2000 (Teklad) containing 56% sucrose, or (2) diet 78053 containing 25% sucrose. The experimental groups of each species were fed one of the cariogenic diets supplemented with 1% dextran either on a daily basis or on alternating weeks. The Keyes' method was used to score caries. The presence of 1% T-10 in either diet did not prevent dental caries in rats ($p > .05$). The daily feeding of diet 2000 + 1% T-10 to 21-day-old hamsters for 60 days did not prevent the formation of dental caries but 1% T-10 did significantly reduce the formation of dental caries in 17-day-old hamsters ($p = .01$). Daily feeding of diet 78053 + 1% T-10 significantly reduced the formation of dental caries in both groups of hamsters ($p = .01$). When young hamsters were fed either diet 2000 + 1% T-10 or diet 78053 + 1% T-10 on alternating weeks, there was a statistically significant reduction in total caries scores ($p = .01$) whether or not the animals received the test diet the first or second week. Plaque accumulation in both rats and hamsters was prevented by the daily feeding of either diet plus 1% T-10. In general the administration of 1% T-10 in the diet prevented dental caries formation in hamsters and plaque accumulation in rats and hamsters.

Supported by NMRDC MR0412002.0041.

American Society for Microbiology, 82nd Annual Meeting, Atlanta, Georgia,
March 7-12, 1982.

1. R. G. ESQUIRE* and I. L. SHKLAIR - "Quantitation of Dental Plaque
Bacteria in Relation to Caries Experience in Young Adults"

*Author presenting paper.

Quantitation of Dental Plaque Bacteria in Relation to Caries
Experience in Young Adults. R. G. ESQUIRE* and I. L. SHKLAIR.
Naval Dental Research Institute, Great Lakes, Illinois 60088

In this study Streptococcus mutans and Lactobacillus were quantified in posterior interproximal dental plaque and related to the dental caries experience of 291 naval personnel with a mean age of 20 ± 2.8 . Plaque samples were collected and transported in 1 ml of 0.5% NaCl, centrifuged, decanted and the pellet weighed. The pellet was resuspended in 1 ml of 0.5% NaCl and 0.2 ml transferred to 1.8 ml 0.5% yeast extract. The bacteria in the yeast extract suspension were diluted and plated on Mitis-Salivarius Bacitracin agar and Rogosa SL agar. Colony counts were recorded as CFU/mg of plaque. The mean counts were: S. mutans, $9.4 \pm 19.28 \times 10^4$ CFU/mg and Lactobacillus, $2.64 \pm 8.96 \times 10^3$ CFU/mg. Caries experience was measured as the sum of decayed, missing and filled tooth surfaces (DMFS). The mean DMFS of the group was 16.49 ± 9.08 . DMFS subgroups above and below this mean were compared in terms of plaque bacterial content. The subgroup above the mean showed significantly higher concentrations of S. mutans ($p < .05$) and Lactobacillus ($p < .01$) than the subgroup below the mean. In the procedure described absolute numbers of bacteria in a specific quantity of dental plaque were related, on a group basis, to dental caries experience.

Supported by NMRDC 62758N MF58.524.012.0028.

National Institute for Dental Research, National Caries Program, Conference on Glucosyltransferases, Sucrose, Glucans and Dental Caries, Louisville, Kentucky, November 15-17, 1982.

1. Lloyd Simonson and Burton Lamberts - "Glucanohydrolases and Control of Glucans"

Many studies have correlated the occurrence of dental lesions with the presence of Streptococcus mutans. The synthesis of extracellular glucans by these bacteria in the presence of sucrose appears to be a specific and necessary prerequisite for the occurrence of dental caries. Although the glucans can be separated into water-soluble and -insoluble fractions that contain both α -1, 6 and α -1, 3-glucosidic linkages, the water-soluble component is rich in α -1, 6 linkages whereas the water-insoluble component is predominantly α -1, 3-linked. Some studies indicate that the glucans may function by promoting bacterial colonization of tooth surfaces. In this regard, the factors that affect initial adsorption of S. mutans onto teeth appear to be quite different from those that interfere with the subsequent accumulation phase.

Some early studies showed that dextranase (α -1, 6 glucan 6-glucanohydrolase) could prevent dental caries in controlled animal experiments. However, conflicting results were observed when the enzymes were used in human clinical trials. It was postulated that this lack of success might have been due to the relatively short period the enzyme was retained in the mouth. We identified and characterized a dextranase from the fungus Fusarium moniliforme (FD) which had a much greater affinity for hydroxyapatite and saliva-coated hydroxyapatite, than a commercial Penicillium-derived dextranase (PD). The FD enzyme was shown to inhibit the initial adsorption of S. mutans in vitro, and was also found capable of preventing the accumulation of S. mutans in the presence of sucrose by its enzymatic activity. The FD was shown to prevent dental caries in hamsters to a greater degree than PD.

This observation prompted studies to develop an improved method for delivering caries-preventive enzymes. We have been pursuing a concept for prolonging enzyme activity in vivo by chemically conjugating glucanohydrolases to molecules which have a high affinity for tooth enamel surfaces. The enzyme-carrier conjugate could act as an artificial plaque-preventing enzyme pellicle, potentially interfering with both initial adsorption and glucan associated accumulation of cariogenic bacteria.

An increasing number of studies now correlate the synthesis of water-insoluble glucan with the cariogenic capacity of S. mutans isolates. Many of these reports suggest that the predominantly α -1, 6-linked "dextrans" are of lesser importance than the predominantly α -1, 3-linked water-insoluble glucans. We have recently reported a new bacterial source of α -1, 3-glucanase as a potential source of enzymatic control of oral glucans. The enzyme was characterized and purified after defining some parameters for optimizing its production. The enzyme is elaborated by a species of Pseudomonas and functions as an endo-hydrolase (endo-1, 3- α -D-glucanase). Like dextranase, the 1, 3-glucanase was able to interfere with the sucrose-mediated increase in accumulation of S. mutans in vitro, even at concentrations below 1 Unit/ml as defined by our assay system. However, once the streptococcal cells have attached to the HA surfaces and formed glucans, much higher concentrations of α -1, 3-glucanase are needed in order to release the cells. The new α -1, 3-glucanase was found to significantly reduce total caries scores in hamsters. These results agree with previous animal studies and certain limited human clinical studies using fungal α -1, 3-glucanase.

Since glucanohydrolase activities primarily interfere with the bacterial accumulation process, we developed an in vitro model to evaluate the role of water-insoluble glucans on cellular accumulation. The model was modified

from the procedure of Clark and Gibbons, 1977. The model measures the quantitative adherence of tritium-labelled oral streptococci to water-insoluble glucans which have been compressed into uniformly sized disks. Data with this method showed that serotypes that synthesize the greatest amount of water-insoluble glucan also had a correspondingly higher degree of accumulation. We could not demonstrate a salivary factor from caries-free subjects which could interfere with cell-to-glucan or glucan-to-glucan accumulation.

Although our studies show that glucanohydrolases can interfere with S. mutans adherence other possible mechanisms may also explain their oral protective properties. For example, recent reports indicate that the water-insoluble glucans may be important as an acid diffusion barrier which could concentrate microbial acids on the tooth surface. In summary, the use of glucanohydrolases, especially α -1, 3-glucanase, appears to be a very specific, safe and promising approach for the control of dental caries and possibly periodontal disease.

Supported by Naval Medical Research and Development Command, Project Number MF5852404B.0027.

American Society for Cell Biology, 22nd Annual Meeting, Baltimore, Maryland,
November 30 - December 4, 1982.

1. B. R. MERRELL* and M. PORVAZNIK - "Lack of Binding between Human Plasma
Fibronectin and Lipopolysaccharides from Oral Anaerobes"

*Author presenting paper.

Lack of Binding between Human Plasma Fibronectin and Lipopolysaccharides from Oral Anaerobes. B. R. Merrell* and M. Porvaznik, Naval Dental Research Institute, Great Lakes, IL 60088.

The purpose of this study was to determine whether human plasma fibronectin (FN) binds specifically to bacterial endotoxins, which are complex lipopolysaccharides (LPS) derived from the outer membrane of Gram negative bacteria. Fibroblast attachment and spreading were used as a functional indication for the presence of active FN. LPS at a concentration as low as 12.5 ng/cm^2 from the oral anaerobe Fusobacterium nucleatum or at 125 ng/cm^2 for Bacteroides melaninogenicus ss. intermedius significantly inhibited ($p < 0.001$) human fibroblasts attachment to cell culture dishes. Incubation of LPS-coated culture dishes with FN at $50 \text{ } \mu\text{g/ml}$ for 15 min at 37°C significantly enhanced ($p < 0.001$) cell attachment, returning it to control levels. However, initial cell attachment was not significantly enhanced by FN, when the LPS-coated culture dishes were pretreated with bovine serum albumin (BSA) at 1 mg/ml in saline for 1 hr at 37°C . Using an enzyme-linked antibody assay (ELISA), we observed that FN did not bind specifically to the LPS from either F. nucleatum or B. melaninogenicus. Enhanced fibroblast attachment to LPS-coated, FN-treated culture dishes was probably the result of nonspecific attachment of FN to the plastic surface in areas not occupied by the globular LPS. BSA pretreatment of the plastic culture dishes masked any available surface other than the LPS for FN to bind. The inability of plasma FN to bind specifically to LPS from oral anaerobes agrees with other studies suggesting that FN does not generally bind to Gram negative bacteria.

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Amalgam restorations; pulp exposure; indirect pulp cap; direct pulp cap; dental emergencies; plaque fluoride; NaF rinsing; caries-free subject; caries-active subjects; salivary peroxidase; third molars; <u>Streptococcus mutans</u> ; saliva; sucrose; insoluble streptococcal glucan; T-10 dextran; glucanohydrolases; human plasma fibronectin; lipopolysaccharides		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
These abstracts are a synopsis of research reported in 1982 by the scientific staff of the Naval Dental Research Institute and collaborators at other research activities. <i>For use in the dental literature</i>		

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